Does Oxidative Stress Involve in Diabetes Mellitus? A Case Study of Lipid Peroxidation, Antioxidants and Lipid Levels in Alloxan Induced Diabetic Rabbits

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Abstract

Whether Oxidative stress is associated with diabetes mellitus and its complications in still a matter for research in recent years. We had assayed for oxidative stress mediated lipid peroxidation product, malondialdehyde (MDA) along with other oxidative stress associated parameters to ascertain and reinforce earlier speculations, reports and debate. We also determined the levels of these parameters as diabetes mellitus progressed. The method of Das and others was used to assay for MDA level while the methods of Tietz, Searcy and Bergquist were used to determine ascorbic acid (vitamin C) and total cholesterol levels respectively. Alloxan at 180mg/kg body weight was used to induce diabetes mellitus in the rabbits which might have resulted from the increased susceptibility of the pancreas to oxidative stress. A significant (P<0.05) increase in the lipid peroxidation product (MDA) was observed in diabetic groups compared to the control. High level of MDA infers increased lipid peroxidation, a condition mediated by oxidative stress. This increase in MDA level continued as diabetic condition progressed to the fifteenth day of the experiment. This could suggest that the condition was aggravated as the duration of diabetes continued. The antioxidant vitamin C level in diabetic rabbits was significantly (P<0.05) lower than in the non diabetic group. Diabetic rabbits total cholesterol level significantly (P<0.05) increased compared to the control. These results indicate inverse relationship between malondialdehyde and ascorbic acid. On the other hand total cholesterol level increased as malondialdehyde level increased. Ascorbic acid counters free radicals and this could lead to its diminution. Oxidative stress could alter lipid metabolism. This may then affect the whole system. This work therefore further reinforces earlier proposals of oxidative stress involvement in alloxan induced diabetes mellitus. Hence alloxan could induce diabetes mellitus by oxidative stress mechanism. Again duration of diabetes matters a lot in drawing conclusion since progression of diabetes aggravated diabetes complications and oxidative stress as observed from the results in the this work. Further work could include studies on the ways of amelioration and prevention of diabetes mellitus and its complications. This may involve incorporating antioxidants to feed diabetic animals and observing the effects.

Keywords: Oxidative stress, lipid peroxidation, malondialdelyde, Vitamin C, Diabetes mellitus.

Introduction

The biochemistry of free radicals is recently evoked. The likely associations of oxidative stress in the pathogenesis of most disease states is now prompting researches into the seemingly implication of oxidative stress in diabetic condition. Thus, the role of oxidative stress in the pathogenesis of diabetic sequel is currently under research and discussion.

Biochemistry of free radical, a novel area in molecular research for disease pathology revolves around the perturbation in the steady state of the metabolic system and the effect of this perturbation on the entire system (Ogugua, 2000). This area of biochemistry is now being looked at as the proximate cause and or exacerbation of disease states. Now for instance, oxidative stress/lipid peroxidation/free radical (free radical biochemistry) is associated with a lot of pathologies (Chiu *et al*, 1982; Fantone and Ward 1982; Wolf *et al.*, 1986; Halliwell *et al.*, 1992; Ogugua, 1994), including those disease states – coronary heart diseases, retinopathy, neuropathy, gastrointestinal tract problem, nephropathy etc. that have some correlations with diabetes mellitus. Some workers have suggested free radical involvement in diabetes pathology and diabetic complications (Cotter *et al.*, 1995; Rema *et al.*, 1995).

Ofordile (1987) hypothesized that alloxan attacks the sulfuhydryl (SH) group of the β cells of the islet of Langerhans which ultimately resulted to diabetes mellitus. The disease has been reported to occur when 80 – 90% of the β cells have been destroyed (Tarn *et al.*, 1987). The involvement of autoimmune reactions (Chung *et al.*, 1992) in the destruction of the β cells and environmental factors, malnutrition, infections, diets and alcohol in diabetes (Oli, 1983; Lehninger, 1987) may further suggest association of oxidant stress in diabetes mellitus, since these factors have been associated with oxidative stress (Oli, 1983; Lehninger, 1987; Chung *et al.*, 1992). Eze *et al.* (1993) have reported the consequences of free radicals on the membrane, as phagocytes attack parasites during infections. The membranes of the host cells including the pancreas may not be spared.

Complications of diabetes - the pathological conditions that result prior to the onset of diabetes, during diabetes and even after diabetes-manifest in various levels in different diabetics. Thus different manifestations may be attributed to genetic factors in individuals. Deposition and elevated levels of cholesterol and low density lipoproteins and other lipids have been suggested to be factors in diabetic complications (Taylor and Gaiu, 1988; Steinberg *et al.*, 1989; Mandini, *et al.*, 2001).

These parameters and most other disease states matter in the complications of diabetes mellitus and some other disease states. These lipids have been associated with atherosclerosis and other cardiovascular diseases (Steinberg et al 1983; Stocker 1993. Antioxidant vitamin C has long been associated with oxidative stress mediated disease states (Frei, 1989; Muma, 1994; Ogugua, 1994; 2000). Its concentration is affected during this condition. The location of Vitamin C in aqueous phase of the membrane plays a significant antioxidant protective role.

Thus, in the present study the question being addressed is "Does oxidative stress actually play roles in alloxan induced diabetes mellitus and its complications. This question will be followed up by estimation of malondialdehyde, glucose, vitamin C and cholesterol levels. It is hoped that the outcome of this study in animal models such as rabbits will illuminate some more areas in the biochemistry of human diabetics and diabetes management.

Materials and Methods

Male albino rabbits six month old, weighing about 1.20 kg, obtained from Umeano rabbitry house, Nsukka were used in the study. They were housed in stainless cages. Two groups (A and B) of five rabbits each were fed with herbs (*Emelia sonchifola* and *Tridax procubene*) and were allowed free access to water. Group B was induced with diabetes by intraperitoneal (IP) injection of alloxan at the concentration of 180 mg/kg body weight in 2 ml normal saline. The group A (non-diabetic group) was given same volume (2 ml) of normal saline without alloxan.

Baseline glucose level was determined by O -toluidine method 24 hours before induction of diabetes. The animals were left for 72 hours (3 days for diabetes to occur and at the end of the three days blood glucose level was again measured by O- toluidine method. The animals with blood glucose level above 9 mmol/L were termed diabetic and thus used for the experiments. Few days were allowed for complications and exacerbation of diabetic condition to set in. Blood glucose level was assayed throughout the experiment to ascertain that the animals were still diabetic before the assay at five days interval for fifteen days

Five milliliters of blood samples were collected from the ear vein of the animals into the heparinized bottles. Plasma was obtained by centrifugation at 1000 rpm for 15 minutes. Plasma lipid peroxidation product (MDA) was estimated by the method of Abro *et al* (1986) and Das *et al* (1990). Vitamin C level was determined by the method of Tietz (1970) while cholesterol levels were assayed by the method of Searcy and Bergquist (1960).

Analyses of variance (ANOVA) of the data were done according to Steel and Torie (1980) and Obi (1986). Results were expressed as means and standard deviations of means.

Results and Discussion

Malondialdelhyde level - marker of oxidative stress mediated lipid peroxidation - has been correlated with the levels of other indices of lipid peroxidation in disease states and oxidative stress conditions (Ogugua 2000; Mandini *et al*, 2001; Uzoegwu and Onwurah, 2003).

Tables 1 and 2 represent the data on glucose level and malondialdehyde level. The increase in glucose level parallel the malondialdehyde level and this result showed that the glucose level increased as the marker (MDA level) of oxidative stress increased.

Table 1: Mean Glucose levels in diabetic and non-diabetic rabbits (mmol/L)

Group	Glucose levels in mmol/L			
	5 days	10 days	15 days	Mean ± SD
A	5.52 ± 0.62	5.63 ± 0.46	5.88 ± 0.73	6.68 ± 0.18
B	16.12 ± 1.42	17.46 ± 0.89	18.24 ± 0.49	17.35 ± 1.02

Table 2: Mean MDA levels in groups A and B (nmol/m/plasma)

Group	Malondialdehyde level in nmol/m/pasma			
	5 days	10 days	15 days	Mean ± SD
A	2.65 ± 0.20	2.66 ± 0.16	2.74 ± 0.15	2.68 ± 0.05
B	6.51 ± 0.24	6.84 ±0.27	7.02 ± 0.15	6.79 ± 0.26

The consequence of excessive blood sugar in diabetic was inferred by the report of Hammels *et al* (1991). This high level of glucose could be as a result of modulation of glucose metabolism in oxidative condition and this resulted to glucose autooxidation which could exacerbate the generation of free radicals (Ting *et al*, 1996). This high level of malondialdehyde in rabbits may suggest that the mechanism of alloxan destruction of the β cells is free radical-mediated which is amplified as diabetic condition and complication progressed. These results corroborate the findings of Graier *et al* (1995) of increased MDA in cells cultured in 20 mmol/L D-glucose. The increased MDA level as glucose level increased in diabetic rabbits lay credence to the report that alloxan stimulated hydrogen peroxide generation (Takasu *et al.*, 1991) which Okamoto (1981) proposed follow the pathway: alloxan → H_2 O_2 → DNA → fragmentation → cell destruction. The fact that oxidative stress mediated lipid peroxidation is involved in the pathogenesis and exacerbation of diabetic state may not be far fetched, as proposed elsewhere (Cotter et al 1995; Rema et al 1995), especially when other indices are put side by side.

Table 3 is the result of the antioxidant vitamin C in both the diabetic and non-diabetic rabbits.

Table 3: Mean vitamin C levels in groups A & B (mg/100ml)

Group	Ascorbic acid (Vitamin C) level in mg/100ml			
	5 days	10 days	15 days	Mean ± SD
A	0.62 ± 0.10	0.61 ± 0.06	0.62 ± 0.11	0.62± 0.10
B	0.34 ± 0.12	0.28 ± 0.06	0.27 ± 0.04	0.30 ± 0.06

The decrease in vitamin C level in diabetic rabbits compared with the control is significantly ($P < 0.05$). Earlier reports (Fantone and Ward, 1982; Frei, 1991; D'aquino and Wilson, 1992) associated decrease in vitamin C level with infections and other disease states. Thus, the low level of this antioxidant vitamin could be a consequence of its capacity to scavenge and mop up free radicals in the system. It has been advocated that vitamin C spares other endogenous antioxidants from consumption by reactive oxygen species (Stocker, 1993). This and other roles of vitamin C in disease states explain the continuous decrease in vitamins level in all the days of the diabetic state. This diminution of the vitamin C level in this work corroborated the observation by Das *et al.* (1990), in malaria patient, another disease associated with the oxidative stress. Thus, the association of low vitamin C level with high MDA level in the present work corroborates reports obtained elsewhere in other diseases and infections (Eze *et al.*, 1993; Muma, 1994; Ogugua, 1994).

The data on Table 4 represent the results of total cholesterol in the plasma of diabetic and non-diabetic animals. Both parameters increased as the oxidative stress marker – MDA – increased and these increases were significant ($P < 0.05$).

Table 4: Total cholesterol levels

Group	Total Cholesterol levels mg/dl			
	5 days	10 days	15 days	Mean ± SD
A	77 ± 9.53	78 ± 12.03	78 ± 12.33	78 ± 0.70
B	184.70 ± 8.68	196.10 ± 14.06	207.30 ± 10.70	196.14 ± 6.08

This could be a result of modification of the mechanism in the metabolism of lipid (cholesterol) by free radicals. Steinberg *et al* (1989) and Stocker (1993) reports of the modification of low-density lipoproteins and cholesterol corroborate the above proposal. The positive correlation of high level of plasma lipids (cholesterol) with increased lipid peroxidation product (MDA) parallels the report on the cytotoxic effect of reactive oxygen which includes peroxidation of membrane lipids, alteration of redox balance, enzyme inactivation and DNA damage (Borg and Schaich, 1984; Slater, 1984). It is thus this peroxidation of the membrane lipids that led to the high levels of lipid in the system. Fatty acids are normally bound but in oxidative perturbation of the system are released as free fatty acids which are components of lipids core prone to attack by free radicals. This propensity of lipids to attack by free radicals has associated oxidative stress mediated lipid peroxidation with most of the coronary heart diseases. Cholesterol is carried in lipoproteins and so oxidation of low-density lipoprotein could result in high level of cholesterol as observed in the present study. This high cholesterol level and its subsequent oxidation is the basis for associating atherosclerosis with diabetes and diabetic complications. Therefore the high level of these lipids in the event of high oxidative stress in diabetes mellitus is a positive expectation as in other disease states.

The study and the findings lay credence to the proposal that free radical are associated with diabetes mellitus. The results further revealed that oxidative stress condition is aggravated as diabetic state progressed. Thus, oxidative stress could be regarded not only the causative agent but also the exacerbating agent in diabetes mellitus. It is likely that the mechanism of alloxan destruction of the β cells is oxidative stress (free radical) - mediated. The amelioration of free radicals and oxidative stress may prevent diabetes mellitus and its complications. Individuals prone to diabetes mellitus may therefore be saved from this condition or have it delayed. It is proposed that mechanism of amelioration and/or prevention of this oxidative onslaught be studied to give this present work some backings. Researching around this is essential since diabetes mellitus cannot be cured but can only be prevented or managed.

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